Development of New Fungicides for Management of Anthracnose and of Diseases of Strawberry, Year 2

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Summary

Our first objectives in developing and registering the new biofungicide natamycin as a pre-plant treatment for managing strawberry anthracnose caused by *Colletotrichum acutatum* have been completed. The Zivion-S label, a 10% SC formulation of natamycin, is currently labeled and available for use on strawberry. The focus of this report is the management of preharvest diseases using natamycin under field conditions. Two of the major diseases of strawberry are gray mold caused by *Botrytis cinerea* and powdery mildew caused by *Podosphaera aphanis*. These pathogens have developed resistance to many registered fungicides, known as multidrug resistance, in many strawberry production areas in California and other parts of the United States. Natamycin is sensitive to ultraviolet (UV) light. We demonstrated that zinc oxide could protect the fungicide from UV degradation. Natamycin-zinc oxide mixtures were highly efficacious against gray mold after artificial inoculation and against natural incidence of powdery mildew. There were mixed results with natural infection of gray mold. Under high disease pressure in 2019, natamycin-zinc oxide treatments significantly reduced gray mold incidence but under low disease pressure in 2018 no differences were observed from that of the control treatment. Increased frequency of sprays, higher concentrations, and/or mixtures with other fungicides may improve efficacy of natamycin.

The white residue on fruit from zinc oxide was reduced at lower concentrations of zinc oxide and may be commercially acceptable. No phytotoxicity was observed. Natamycin was specifically selected to combat fungicide resistance. Despite use for many years in the food industry, no resistance to natamycin has ever been reported in filamentous fungi. Additionally, we demonstrated that Switch® (i.e., fludioxonil/cyprodinil) and a new fungicide pre-mixture, Miravis® Prime (i.e., pydiflumetofen/fludioxonil), have high efficacy against gray mold. Quadris Top® (i.e., Azoxystrobin/difenoconazole) and Merivon® (i.e., pyraclostrobin/fluxapyroxad) were highly effective against powdery mildew.

Introduction

In developing new fungicides for other crops, we identified natamycin as a broad-spectrum, protective, postharvest fungicide for numerous fruit decays. Natamycin has been recently registered as a postharvest treatment of citrus and stone fruits in the United States. The compound is classified by the EPA as a bio-fungicide because it is a natural fermentation product of the bacterium *Streptomyces natalensis* and other species of *Streptomyces*. Compounds classified as bio-pesticides are exempt from tolerance in the US, Canada, and Mexico.

Natamycin is effective against many fungi in the Ascomycota (e.g., *Botrytis*, *Colletotrichum*, and *Podosphaera* spp.) and Zygomycota (e.g., *Rhizopus stolonifer*) phylum that are pathogens of strawberry, and has a minimum inhibitory concentration of <10 ppm for most of these organisms. The compound has been used in the food industry as a preservative of dairy products (e.g., cheese) and dried meats for over 30 years. During this time, no resistance in target organisms including *Penicillium* spp. has ever been reported. This renders natamycin low risk for resistance development.

In preliminary studies the spring of 2016-18, we identified natamycin as a highly effective pre-plant dip for managing anthracnose of strawberry, similar to Switch® (cyprodinil, fludioxonil) and Abound® (azoxystrobin), and no phytotoxicity was observed at the lower rates. Natamycin was also very effective when plants were inoculated with azoxystrobin-resistant isolates of the pathogen. The registrant of natamycin, DSM, has obtained a biofungicide registration for natamycin (this means it has obtained tolerance exemption) and it is labeled as Zivion-S, a 10% suspension concentrate (SC) formulation. Additionally, DSM has submitted a request to the National Organic Standards Board (NOSB) to obtain organic status of the fungicide in the US (Canada currently lists natamycin as organic).

The goal of this part of our research was to evaluate the effectiveness of natamycin as a preharvest treatment against postharvest gray mold and against preharvest powdery mildew. For this, field plantings were established for evaluation of fungicide efficacy against gray mold; whereas greenhouse experiments were conducted to evaluate fungicide performance against powdery mildew.

Objectives

- Determine if zinc oxide can prevent UV light degradation of natamycin
- Evaluate the efficacy of natamycin in the field against gray mold and in the greenhouse against powdery mildew
- Compare the efficacy of natamycin + zinc oxide to conventional fungicides

Materials and Methods

Gray Mold Field Trials

Strawberry cultivars 'Fronteras' and 'Monterey' were established in a field at the University of California Riverside Agricultural Operations experiment station near campus in December 2018. Strawberry plants were planted 30 cm (1 ft) apart in a double row on a raised bed covered with plastic tarp (i.e., mulch similar to commercial plantings). Fungicides were applied using backpack sprayers at 1400 L/Ha (150 gal/A) beginning at bloom and repeated every seven days for three to four applications for the three experimental trials in 2018 (two trials) and 2019 (one trial). The experiment was a Randomized Complete Block Design with each treatment replicated four times.

Seven days after the final application, fruit were collected and inoculated with a drop of 50,000 conidia/ml of *B. cinerea*. After seven days storage at 20ºC, fruit were evaluated for incidence of gray mold originating from the point of artificial inoculation and for natural infection.

Powdery Mildew Greenhouse Experiments

Plants of cultivar 'Splendor' were established in pots filled with UC Planting mix in the greenhouse. Healthy 'Splendor' plants were placed adjacent to diseased plants showing severe powdery mildew symptoms. Upper and lower surfaces of each leaf were treated once with fungicides using a hand sprayer (six plants per treatment). After 18 days, the lower leaf surface of each of three leaves of the same approximate age from each plant was evaluated for powdery mildew using the following severity rating: 0=No disease, 1=1 to 10%, 2=11 to 25%, 3=26-50%, and 4=51-100% diseased area. The experiment was repeated and data were averaged for both experiments.

Results and Discussion

Zinc oxide protected natamycin from UV degradation, preserving activity against gray mold and powdery mildew. Natamycin + zinc oxide treatments at the rates tested were highly effective against gray mold after artificial inoculation (Figures 1 & 2) and against natural infection of powdery mildew (Figures 3 & 4). Mixed results were obtained with natural infection of gray mold (Figure 1). Increased application frequency, higher concentrations, and/or mixtures with other fungicides may improve the efficacy of natamycin. The white residue on fruit and leaves (Figure 4) from zinc oxide was reduced using lower concentrations and may be commercially acceptable. This study demonstrates that natamycin can be formulated with UV light protectants to improve the performance of the fungicide for foliar applications for field or greenhouse use. The registrant is considering pre-mixtures with zinc oxide but tank mixtures may also be a grower strategy once the product is labeled for foliar use against gray mold and powdery mildew, in addition to the pre-plant label for dips against strawberry anthracnose.

Studies to potentially expand the pre-plant dip label to include control of Botrytis 'box rot' or decay of strawberry plants that develops in cold storage and shipment of strawberry transplants are the next step in developing natamycin for the California strawberry industry.

2018 Experiment (cv. 'Fronteras')

Treatment	Rate
Untreated Control	---
Natamycin	500 µg/ml
Natamycin + Zinc Oxide	500 µg/ml + 5.0%
Pydiflumetofen + Fludioxonil	47 µg/ml + 78 µg/ml
Cyprodinil + Fludioxonil	262 µg/ml + 175 µg/ml

2018 Experiment (cv. 'Monterey')

Treatment	Rate
Untreated Control	---
Natamycin	500 µg/ml
Natamycin + Zinc Oxide	500 µg/ml + 5.0%
Pydiflumetofen + Fludioxonil	47 µg/ml + 78 µg/ml
Cyprodinil + Fludioxonil	262 µg/ml + 175 µg/ml

2019 Experiment (cv. 'Monterey')

Treatment	Rate
Untreated Control	---
Natamycin	500 µg/ml
Natamycin + Zinc Oxide	500 µg/ml + 0.5%
Cyprodinil + Fludioxonil	262 µg/ml + 175 µg/ml

* Bars with the same letters are not significantly different using GLM and Least Significant Difference (LSD) procedures (SAS ver. 9.4).

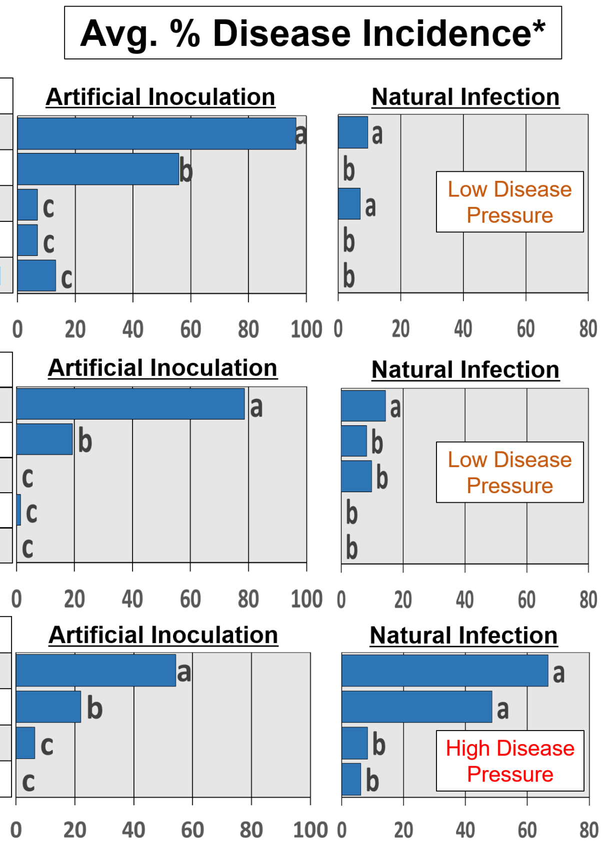

Figure 1. Efficacy of natamycin and natamycin mixtures with zinc oxide as compared to standard commercial and experimental fungicides on cvs. 'Fronteras' and 'Monterey' in 2018 and 2019 for management of gray mold of strawberry. Fruit were artificially inoculated with conidia of *B. cinerea* (50,000 conidia/ml) or were evaluated for the natural incidence of gray mold after incubation for seven days at 20ºC (68ºF).

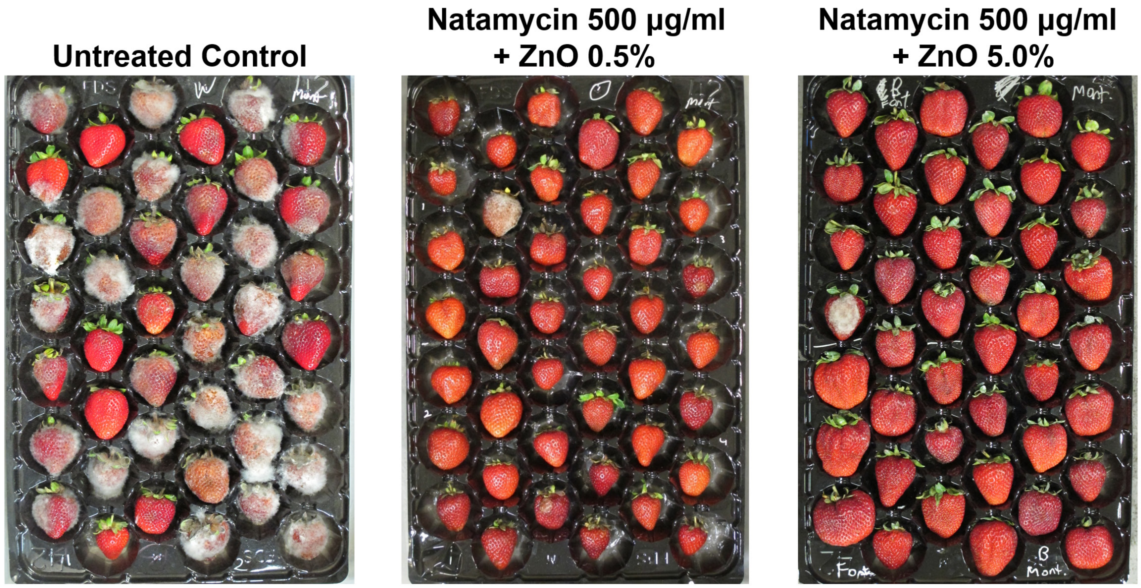

Figure 2. Efficacy of natamycin and natamycin mixtures with zinc oxide (0.5% and 5.0%) as compared to the untreated control on cv. 'Fronteras' for management of gray mold of strawberry. Fruit were artificially inoculated with conidia of *B. cinerea* (50,000 conidia/ml) or were evaluated for the natural incidence of gray mold after incubation for seven days at 20ºC (68ºF).

Treatment	Rate
Untreated Control	---
Natamycin	1000 µg/ml
Natamycin + Zinc Oxide	1000 µg/ml + 1.0%
Azoxystrobin + Difenoconazole	133 µg/ml + 83 µg/ml
Fluxapyroxad + Pyraclostrobin	122 µg/ml + 122 µg/ml

* Bars followed by the same letter are not significantly different based on MIXED model procedures (SAS version 9.4).

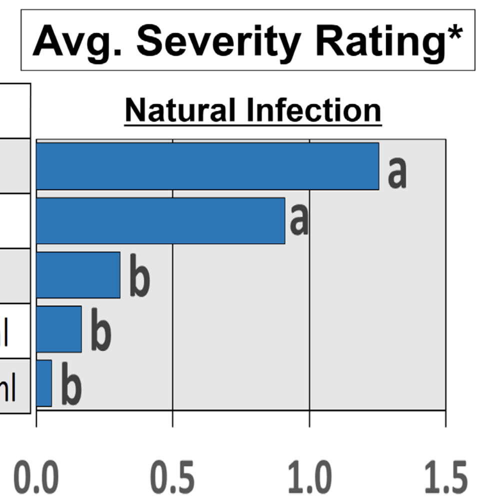

Figure 3. Efficacy of natamycin and natamycin mixtures with zinc oxide as compared to standard commercial fungicides, Quadris Top® (azoxystrobin/difenoconazole) and Merivon® (fluxapyroxad/pyraclostrobin) on cv. 'Splendor' in 2018 for management of natural incidence of powdery mildew of strawberry in greenhouse studies.

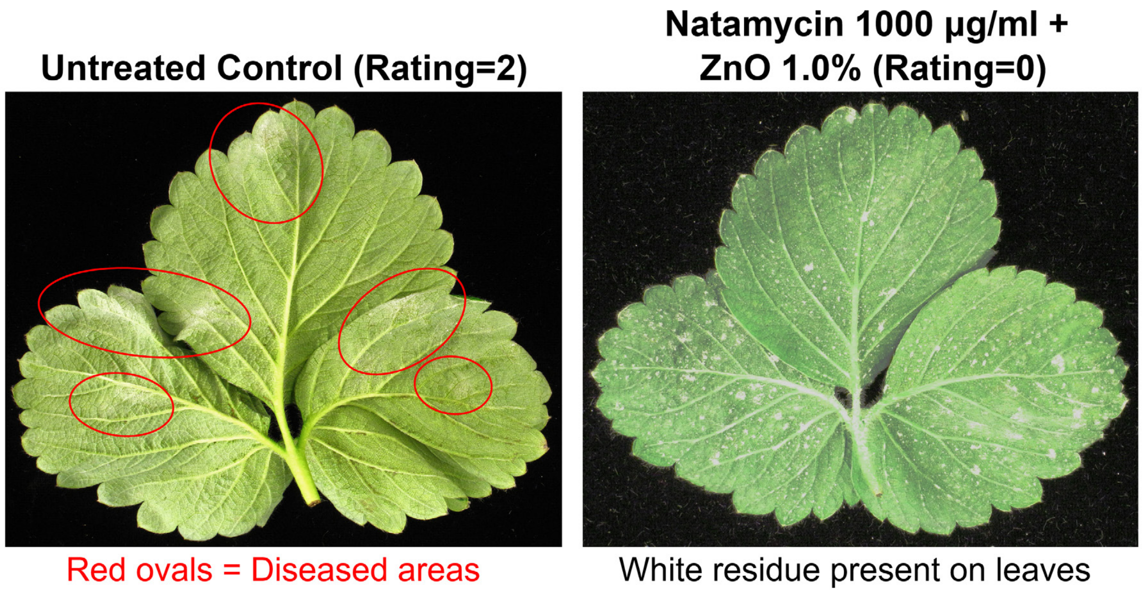

Figure 4. Symptoms of powdery mildew on untreated 'Splendor' leaflets (left) and on leaflets treated with a natamycin (1,000 ppm) and zinc oxide (1%) mixture (right) in a greenhouse study.

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References

- Adaskaveg, J. E., and H. Förster. 2015. New postharvest decay control treatments that are exempt from residue tolerances in the United States. Pages 299- 306, in Proc. Vth International Conference Postharvest Unlimited. Eds.: G.A. Manganaris et al., Acta Hort. 1079, ISHS 2015.Jay, C.N., J.v. Cross, and C. Burgess. 2004. The relationship between populations of European tarnished plant bug (*Lygus rugulipennis*) and crop losses due to fruit malformation in everbearer strawberries. Crop Protection 23: 825-834. doi: http://dx.doi.org/10.1016/j.cropro.2004.01.002.
- Adaskaveg, J. E., and G. J. Holmes. 2018. Efficacy of natamycin as a pre-plant dip treatment for the management of anthracnose of strawberry. California Strawberry Commission Annual Production Research Report 2017:121-132.
- Haack, S. E., K. I. Ivors, G. J. Holmes, H. Förster, and J. E. Adaskaveg. 2018. Natamycin, a new biofungicide for managing crown rot of strawberry caused by QoI-resistant Colletotrichum acutatum. Plant Disease 102:1687-1695.
- Maas, J. L. 1984. Compendium of Strawberry Diseases. American Phytopathological Society, St. Paul, MN.